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Research Article

Polygonatum cyrtonema Hua Polysaccharides with Antiaging and Stress Resistance Efficacies in *Caenorhabditis elegans*

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Polygonatum cyrtonema Hua, a source of Polygonati rhizoma, possesses multiple health-promoting properties and is widely used in food and medicine. This study aimed to elucidate the lifespan promotion abilities and potential mechanism of action of *P. cyrtonema* Hua polysaccharides (PCHPs) on *Caenorhabditis elegans*. The results revealed that PCHP had a molecular weight distribution of 8.77×10^3–1.84×10^6 Da and comprised Man, GalA, Glu, Gal, Xyl, and Ara in a molar ratio of 13.8 : 3.5 : 22.7 : 2.7 : 1 : 1.3. PCHP (5.0 mg/mL) markedly increased the lifespan of *C. elegans* by 15.78% ($p < 0.001$), improved motility, and increased the resistance to heat stress, UV irradiation, and oxidative stress. PCHP also promoted healthy aging by attenuating the accumulation of reactive oxygen species (ROS) and lipofuscin. Furthermore, PCHP upregulated the expression of oxidative stress- and aging-related genes, promoted the migration of DAF-16 into the nucleus, and exerted no effect on the lifespan of *daf-2*, *daf-16*, *age-1*, *skn-1*, and *sir-2.1* mutants. These results indicate that the transcription factors DAF-16, SIR-2.1, and SKN-1 are required for PCHP-mediated lifespan extension in *C. elegans*. In conclusion, PCHP has the potential to attenuate the effects of aging, which can lay the foundation for aging treatment research with *P. cyrtonema*.

1. Introduction

Polygonatum cyrtonema Hua is a medicinal plant of the genus *Polygonatum*, which is mainly distributed in western China and is widely used as a functional food and medicine [1]. As one of the main sources of Polygonati rhizoma (*Huangjing* in Chinese), its rhizomes are commonly employed for treating diabetes, feebleness, fatigue, loss of appetite, and lung problems [2, 3]. In traditional Chinese medicine clinical practice, Polygonati rhizoma has been used as medicine for over 2000 years and has high nutritional values with multiple effects on replenishing the spleen, moistening the lungs, boosting qi, nourishing yin, and benefiting the kidneys [3]. Previous studies have suggested that the multiple biological activities of Polygonati rhizoma can be attributed to various components, including polysaccharides, steroidal saponins, triterpenoid saponins, flavonoids, phenylpropanoids, and alkaloids [4–6]. Among them, polysaccharides are the most important bioactive compounds in Polygonati rhizoma.

The polysaccharide level is representative of the quality of Polygonati rhizoma. Polygonati rhizoma polysaccharides (PRPs) have been reported to possess numerous biological and pharmacological activities, including antioxidative, antiaging, anti-inflammatory, antidiabetic, and anticancer properties [7, 8]. PRPs also have immune-enhancing, neuroprotective, and blood fat reducing effects [9]. *P. cyrtonema* Hua polysaccharides (PCHPs), the significant bioactive components in *P. cyrtonema,* have gained more attention for their various biological activities [8, 10]. However, differences in the processing and source of Polygonati rhizoma result in changes in the molecular weight

distribution and monosaccharide composition of polysaccharides, which further affect its activities [9, 11]. Furthermore, its biological activity depends on other factors, such as the structure, location, type, and spatial configuration of the glycosidic bond, as well as the acetyl group attached to the residue [12, 13]. The polysaccharides from raw *P. cyrtonema* mainly consist of neutral polysaccharides with a molecular weight fraction of 2.09–8.5 kDa, and the monosaccharide composition mainly comprises of Gal, GalA, Man, Rha, Ara, and Glc, in a molar ratio of 3 : 6 : 16 : 1 : 3 : 8 [13–15]. The molecular weight fraction of polysaccharides from steamed rhizomes increased to 1200–4400 kDa, and the molar ratio changed to 7.3 : 5 : 6 : 1 : 2.4 : 1 after steaming and drying for one cycle [13–15]. Steam-bask and wine stewing are traditional methods for processing Polygonati rhizoma; presently, the steam-bask process has been partly replaced by steam baking. Raw rhizomes are mainly processed by steaming to reduce toxicity and enhance efficacy. Meanwhile, the steam processing can reduce irritation in the throat and tongue [16]. Polysaccharides, both from raw Polygonati rhizoma or processed by wine stewing, have the effects on delaying aging [11].

Caenorhabditis elegans is a classic model for research on aging based on the advantages of its short lifespan, easy cultivation, broad availability of mutants, and highly conserved longevity genes and signaling pathways between *C. elegans* and humans [17]. Several signaling pathways that extend lifespan have been identified, among which, the insulin/IGF signaling (IIS) pathway is a thoroughly studied pathway involved in stress-activated protective responses and aging [18]. Associated with the IIS pathway, DAF-16/FoXO, SIR 2.1/SIRT, and SKN-1/Nrf2 are essential transcription factors that can extend the lifespan and improve stress resistance in *C. elegans* [18, 19]. Polysaccharides can increase stress resistance and extend the life of *C. elegans* by modulating the IIS pathway [20–22]. However, there are no reports on the antiaging effects of *P. cyrtonema* on *C. elegans*. In particular, the antiaging effects of PCHP and the underlying mechanism in *C. elegans* are unclear. Considering that the rhizome from *P. cyrtonema* processed by steam baking is widely consumed as a functional food and medicine, we explored the antiaging effects of polysaccharides from *P. cyrtonema* after steam baking.

In this study, *C. elegans* was used to investigate the effects of PCHP on the prolongation of lifespan and to uncover the molecular mechanisms of antiaging and stress resistance. The lifespan, pharyngeal pumping, reproductive capacity, locomotive behavior, intestinal lipofuscin, intracellular reactive oxygen species (ROS), and stress-related indicators were evaluated. Furthermore, the genes and insulin-like signaling pathways associated with oxidative stress were assessed.

2. Materials and Methods

2.1. Materials and Reagents. The processed rhizomes of *P. cyrtonema* (produced in 2020, steamed and roasted for one cycle) were purchased from Jinzhai Runyuan Biotechnology Co., Ltd. (Lu'an, China) and stored at −20°C prior to use.

The dialysis bags were purchased from YuanYe Bio-Technology Co., Ltd. (Shanghai, China). All chemicals and reagents were purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China), Energy Chemical (Shanghai, China), and Chengdu Kelong Chemical Reagent Co., Ltd. (Chengdu, China). The chemicals and reagents used in this study were of analytical grade.

2.2. Preparation of Polygonatum Cyrtonema Hua Polysaccharides and Molecular Weights and Analysis of Compositional Monosaccharides. Dry rhizomes of *P. cyrtonema* were ground and passed through a 60-mesh sieve. The samples were extracted using 90% ethanol via ultrasonication at 250 W for 1 h. After removing the lipid-soluble ingredients, the residues were dried in a heated air-dryer at 50°C and extracted using water via ultrasonication at 250 W for 1 h. Extraction was repeated three times. The water extract was concentrated, and ethanol was added until the ethanol concentration reached 80%. Then, samples were stored at 4°C overnight to obtain the crude polysaccharides via suction filtration. The precipitates were dissolved in ultrapure water and deproteinized using the Sevag method [23]. After protein removal, the polysaccharides were further purified for 24 h using a dialysis bag with a molecular weight cutoff of 7 kDa, and the purification step was repeated six times. The solution that dialyzed in the bag was concentrated and lyophilized to obtain PCHP for use in subsequent experiments. The molecular weight distribution and monosaccharide composition of PCHP were analyzed using HPSEC-RID-MALLS (DAWN HELEOS, Wyatt Technology, USA) and HPLC (ACQUIRED Arc., Waters, USA) according to the previous methods with some modifications [24, 25].

2.3. Caenorhabditis elegans Strains and Maintenance Conditions. The *C. elegans* strains used in this study were obtained from the Caenorhabditis Genetics Center (CGC), University of Minnesota (Minneapolis, MN, USA), including wild-type N2, *daf-16 (mu86)*, *daf-2 (e1370)*, *TJ356 (zIs356 IV)*, *skn-1 (zu135)*, *sir-2.1 (ok434)*, and *age-1 (hx546)*.

All strains were cultured on nematode growth medium (NGM) plates at 20°C (unless stated otherwise), and *Escherichia coli* OP50 was provided as a food source.

2.4. Lifespan Assay. Fifty synchronized L4-stage worms were placed onto NGM plates containing different concentrations of PCHP (2.0, 5.0, and 10.0 mg/mL) or without PCPH (control) and cultured at 20°C (from day 0). The living worms were transferred to a new plate at every alternate day and recorded until all worms were dead (criterion: if the worm had no response when it was slightly touched with a picker, it was marked as dead) [18]. All lifespan assays were performed independently in triplicate.

2.5. Reproduction Assay. Synchronized L4-stage worms were individually transferred to a new NGM plate with or without 5.0 mg/mL PCHP daily during the reproduction period. The total number of offspring for each parental worm was counted by summing the hatched progeny produced in the entire study period [11].

2.6. Pharyngeal Pumping Assay. Age-synchronized N2 worms were treated with or without 5.0 mg/mL PCHP. After 4 or 8 days of treatment, the pharyngeal pumping rate was determined for 10 individual worms per 30 s [20]. Each assay was performed in triplicate.

2.7. Motility Assay. Twenty L4-stage worms were transferred to NGM plates containing 5.0 mg/mL PCHP and transferred to new plates at every alternate day. Mobility was observed on days 7 and 10. The motility classes of the worms were determined by scoring their locomotion phenotypes. Class a: worms moved spontaneously and smoothly without touching; Class b: worms moved uncoordinatedly or slowly; and Class c: worms moved their head or tail scarcely after prodding but were clearly alive [26].

2.8. Measurement of Reactive Oxygen Species (ROS). After exposure to 5.0 mg/mL PCHP for 8 days, intracellular ROS levels in worms were measured using 1000 μM 2′,7′-dichlorodihydrofluorescein diacetate [27]. The plate was incubated at 37°C for 2 h. Worms without PCHP treatment were used as controls. Fluorescence intensity was measured using a fluorescence microscope (excitation 485 nm and emission 535 nm) and analyzed using ImageJ. The assay was performed in triplicate, with 20 randomly selected worms per group.

2.9. Lipofuscin Assay. After exposure to 5.0 mg/mL PCHP for 8 days, the fluorescence intensity of worms was measured using a fluorescence microscope (excitation 365 nm and emission 420 nm) [20]. Fluorescence intensity was analyzed using ImageJ. Worms treated without PCHP were used as controls. Twenty worms were examined in each group, and the assay was performed in triplicate.

2.10. Stress Resistance Assays. Synchronized L4 larvae were transferred to NGM plates (both with and without 5.0 mg/mL PCHP) at 20°C. For heat stress assay, the worms (4-days-old) were exposed at 37°C [27]. The number of alive worms was counted every hour until all had died. For the UV irradiation assay, 4-day-old worms were irradiated with 254 nm UV light [27]. The number of surviving worms was recorded every day until the last worm had died. For the oxidative stress assay, 4-day-old worms were transferred to a new NGM plate containing 50 mM paraquat with or without 5.0 mg/mL PCHP [20]. Surviving worms were recorded daily until the last worm died. Stress resistance assays were performed in triplicate.

2.11. DAF-16::GFP Localization Assay. Synchronized L4-stage*TJ356 (zIs356 IV)* worms were transferred onto NGM plates with or without 5.0 mg/mL PCHP for 8 days. Worms without PCHP treatment were used as controls. After mounting on 2% agarose pads, the DAF-16::GFP distribution was observed under a fluorescence microscope (excitation 485 nm and emission 535 nm) and analyzed using ImageJ [27]. The assay was performed in triplicate, with 20 randomly selected worms per group.

2.12. Quantitative RT-PCR Assay. Total RNA was extracted from approximately 1,000 worms that were treated with or without 5.0 mg/mL PCHP for 8 days using TRIzol reagent, and the RNA was converted to cDNA using the PrimeScript™ Reagent Kit [11]. The RT-PCR was carried out using a Bio-Rad Minioption™ Real Time PCR Detection System (Bio-Rad, Hercules, CA, USA) with SYBR green fluorescence dye (Vazyme Biotech Co., Ltd., Nanjing, China). Actin was used as a reference gene, and the data were analyzed using the $2^{-\Delta\Delta Ct}$ method. The primers used in the experiment are listed in Table 1.

2.13. Statistical Analysis. The results were analyzed and presented as mean ± standard deviation (SD) ($n = 3$). The survival curve of *C. elegans* was analyzed using GraphPad Prism version 9. One-way ANOVA with Duncan's multiple range test or a Student's *t*-test was used to analyze the differences between the experimental and control groups. A p value less than 0.05 was considered as statistically significant ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$).

3. Results and Discussion

3.1. Molecular Weight Distribution and Monosaccharide Composition of PCHP. As shown in Figure 1 and Table 2, the HPSEC chromatogram of PCHP was divided into five peaks with a molecular weight distribution of 8.77×10^3–1.84×10^6 Da. HPLC analysis of the monosaccharide composition revealed that the PCHP used in the experiment mainly comprised Man, GalA, Glu, and Gal, with small amounts of Xyl and Ara (Figure 2). Six monosaccharide standards were employed to establish standard curves (Figure S1). The molar ratio of Man, GalA, Glu, Gal, Xyl, and Ara in PCHP

Table 1: Primer sequences for qRT-PCR.

Gene	Forward primer	Reverse primer
actin-1	CCAGAAGAGCACCCAGTC	TGATGTCACGGACGATTT
daf-2	GCCCGAATGTTGTGAAAACT	CCAGTGCTTCTGAATCGTCA
daf-16	ATCGTGTGCTCAGAATCC	ATGAATATGCTGCCCTCC
sod-3	AGAACCTTCAAAGGAGCTGATG	CCGCAATAGTGATGTCAGAAAG
sir-2.1	TGGCTGACGATTCGATGGAT	ATGAGCAGAAATCGCGACAC
skn-1	CACGCCGTCAGCGAAGTA	ATGCTCGGTGAGTATTGG

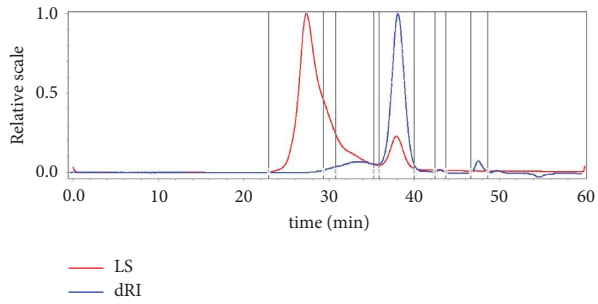

Figure 1: HPSEC-RID-MALLS chromatogram of PCHP (consisting of five peaks: peak-1 (22.9–29.4 min, peak-2 (30.7–35.2 min), peak-3 (35.8–40.0 min), peak-4 (42.4–43.7 min), and peak-5 (46.7–48.6 min)).

Table 2: The molecular weight distribution of PCHP.

No.	Peak-1	Peak-2	Peak-3	Peak-4	Peak-5
Molecular weight	1.84×10^6	6.20×10^4	7.78×10^3	4.67×10^4	8.77×10^3
Error	±1%	±2%	±2%	±18%	±26%

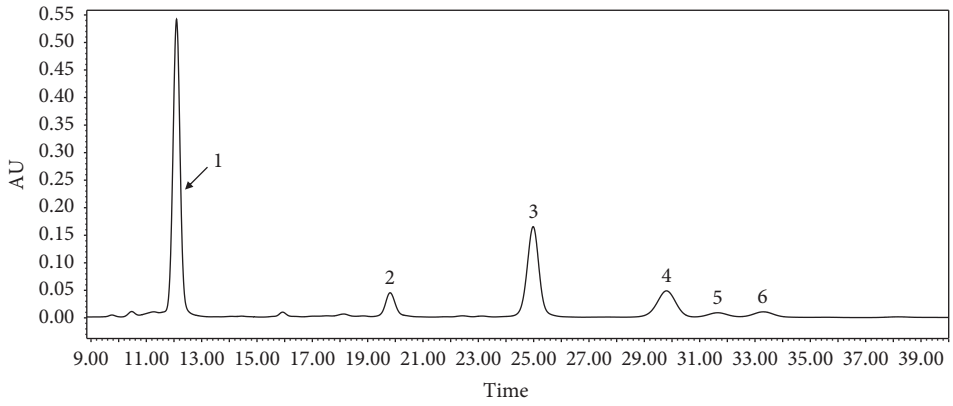

Figure 2: HPLC analysis of monosaccharide derivatives after complete acid hydrolysis of PCHP (1: Man-derivative; 2: GalA-derivative; 3: Glu-derivative; 4: Gal-derivative; 5: Xyl-derivative; 6: Ara-derivative).

was 13.8 : 3.5 : 22.7 : 2.7 : 1 : 1.3. Molecular weight and monosaccharide composition of PCHP were affected by different processing methods [14, 15]. Hydrolysis of polysaccharides resulted in changes of chemical structure and monosaccharide composition during the processing [28]. However, the main monosaccharides of PCHP have not changed according to the previous reports, they were Man, GalA, Glu, and Gal [15, 29]. This study also provides strong confirmation of the main monosaccharide of PCHP. Although the monosaccharide composition of PCHP identified in this study was almost consistent with that of the previous studies [15], the proportion was completely different, which may be attributed to the samples being obtained from different geographical locations or changes in the processing conditions.

3.2. Effect of PCHP on the Lifespan of Wild-TypeC. elegans. To evaluate the antiaging properties of PCHP, the wild-type N2 worms were cultured on NGM plates containing PCHP with different concentrations at 20°C. The mean lifespan of *C. elegans* was greatly prolonged after treatment with PCHP

(2.0, 5.0, and 10.0 mg/mL), which increased to 17.96 ± 0.48, 19.42 ± 0.20, and 18.07 ± 0.44 days, respectively, compared with the control group (16.77 ± 0.04 days) (Figure 3 and Table 3). At 5.0 mg/mL PCHP, the largest mean lifespan extension was displayed at 15.78%. Meanwhile, the extension of mean lifespan was increased by 7.07% and 7.71% at 2.0 and 10.0 mg/mL PCHP, respectively. The survival rate of worms treated with 10.0 mg/mL PCHP was higher than that of worms treated with 2.0 mg/mL PCHP before the middle and later stages of life. However, 10.0 mg/mL PCHP accelerated aging at a later life stage. This suggests that an appropriate concentration of PCHP is crucial from the perspective of antiaging, and its effects may depend on improving the energy metabolism of the mitochondria or reducing the expression of DNA polymerase γ [30]. The results indicated that 5.0 mg/mL PCHP significantly promoted the mean lifespan of *C. elegans*; therefore, 5.0 mg/mL PCHP was used to treat worms for comparison with the untreated worms in all subsequent assays.

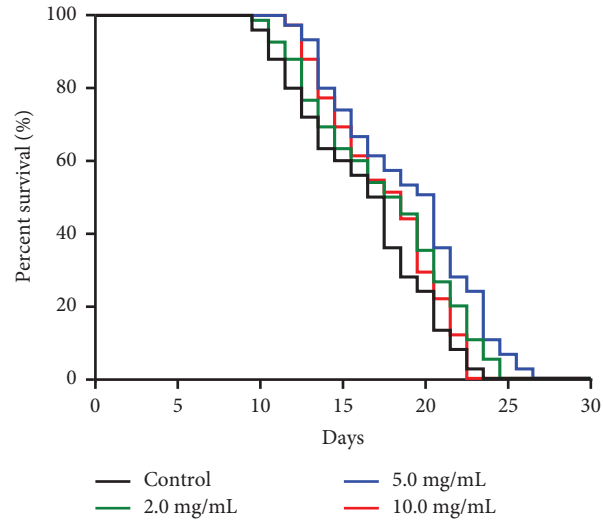

Figure 3: The survival rate curves of wild-type*C. elegans* treated with 2.0, 5.0, and 10.0 mg/mL PCHP at 20°C.

3.3. Effects of PCHP on the Reproduction, Pharyngeal Pumping, and Locomotion of Wild-TypeC. elegans. Studies have demonstrated that several physiological responses accompany the aging process of *C. elegans*, including reproductive behavior, pharyngeal pumping, and locomotion capacity, which gradually decline with age [31]. To assess the effect of PCHP on reproductive ability, which is a significant indicator used to understand the aging process, the number of eggs was used as a parameter to evaluate reproduction of wild-type*C. elegans*. The results showed that treatment with 5.0 mg/mL PCHP did not have a significant influence on the reproductive capacity of the worms compared to that in the control group (Figure 4(a)).

The food intake of *C. elegans* was estimated by counting pharyngeal pumping. We observed that 5.0 mg/mL PCHP had no effect on the pharyngeal pumping rate on days 4 and 8 (Figure 4(b)). The results indicated that 5.0 mg/mL PCHP did not improve the swallowing muscle of the worms, suggesting that the effect of PCHP on life extension might not depend on diet restriction.

To elucidate the effects of PCHP on the motility of *C. elegans*, wild-type N2 worms treated with 5.0 mg/mL PCHP were evaluated. As shown in Figure 4(c), the motility of worms was significantly improved at days 7 and 10, representing two developmental stages (early-middle and middle stages). In the two stages, the proportion of worms that moved spontaneously and smoothly (Class a) was considerably higher than that in the control, especially in the middle stage. Compared to the control group, motility was 28.49% and 75.82% higher in Class a at days 7 and 10, respectively. There was also an improvement in Class b in the middle stage, which was 50.82% higher than that in worms treated without PCHP.

Improving the health cycle is important for delaying entry into the aging stage. In addition to lifespan extension of PCHP, healthy aging is also very significant. Reproductive behavior, pharyngeal pumping, and locomotion capacity are the main healthy indicators used as significant consideration during aging [32]. Our findings revealed that PCHP did not improve physiological functions, such as reproduction and pharyngeal pumping. Therefore, treatment with 5.0 mg/mL PCHP showed no reproductive toxicity on *C. elegans* and had no improvement in its swallowing muscle. The results revealed that PCHP did not cause damage to *C. elegans* while extending lifespan. However, it significantly enhanced motility, indicating that PCHP can promote muscle health in worms. Based on these observations, the effect of PCHP on lifespan extension, at least in part, was ascribed to the improvement of locomotion capacity.

3.4. Effects of PCHP on ROS and Lipofuscin Accumulation of Wild-TypeC. elegans. According to the theory that the aging process is dominated by free radicals, intracellular ROS can accelerate aging by inducing oxidative stress [33]. To investigate whether PCHP could reduce the ROS levels in *C. elegans*, we measured the intracellular ROS levels on day 8. The intracellular ROS level in *C. elegans* treated with 5.0 mg/mL PCHP was effectively decreased by 40.01% compared with that in the control group (Figure 5(a)). This study demonstrated that the effect of PCHP on lifespan extension could be partly associated with the intracellular ROS scavenging ability of PCHP in *C. elegans*, suggesting that PCHP could stimulate the antioxidant defense system.

Accumulation of lipofuscin is a significant feature that accompanies aging. The effect of PCHP on lipofuscin accumulation in *C. elegans* was also assessed. In contrast to the untreated worms, the lipofuscin fluorescence intensity in worms treated with 5.0 mg/mL PCHP was significantly decreased by 28.78% on day 8 (Figure 5(b)), indicating that PCHP could significantly decrease the accumulation of lipofuscin in *C. elegans*, which might be associated with the ROS scavenging effect.

The balance between production and consumption of ROS in organism is crucial, otherwise, it will cause oxidative stress, which is related to a variety of diseases [12, 34].

Table 3: Effects of PCHP on the lifespan of *C. elegans* at 20°C.

Strain	Group	Mean lifespan ± SD (days)	Change in lifespan[a] (%)
N2	Control	16.77 ± 0.04	
N2	PCHP (2.0 mg/mL)	17.96 ± 0.48**	7.07
N2	PCHP (5.0 mg/mL)	19.42 ± 0.20***	15.78
N2	PCHP (10.0 mg/mL)	18.07 ± 0.44**	7.71
daf-2 (e1370)	Control	37.96 ± 1.14	
	PCHP (5.0 mg/mL)	26.55 ± 0.98***	−30.06
daf-16 (mu86)	Control	16.32 ± 0.78	
	PCHP (5.0 mg/mL)	14.58 ± 0.26*	−10.62
age-1 (hx546)	Control	24.05 ± 0.46	
	PCHP (5.0 mg/mL)	23.18 ± 0.95	−3.60
skn-1 (zu135)	Control	18.84 ± 0.53	
	PCHP (5.0 mg/mL)	18.91 ± 0.07	0.37
sir-2.1 (ok434)	Control	16.82 ± 0.32	
	PCHP (5.0 mg/mL)	16.34 ± 0.21	−2.9

Note: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, significantly different when compared to the control group. [a]Percentage is relative to the control.

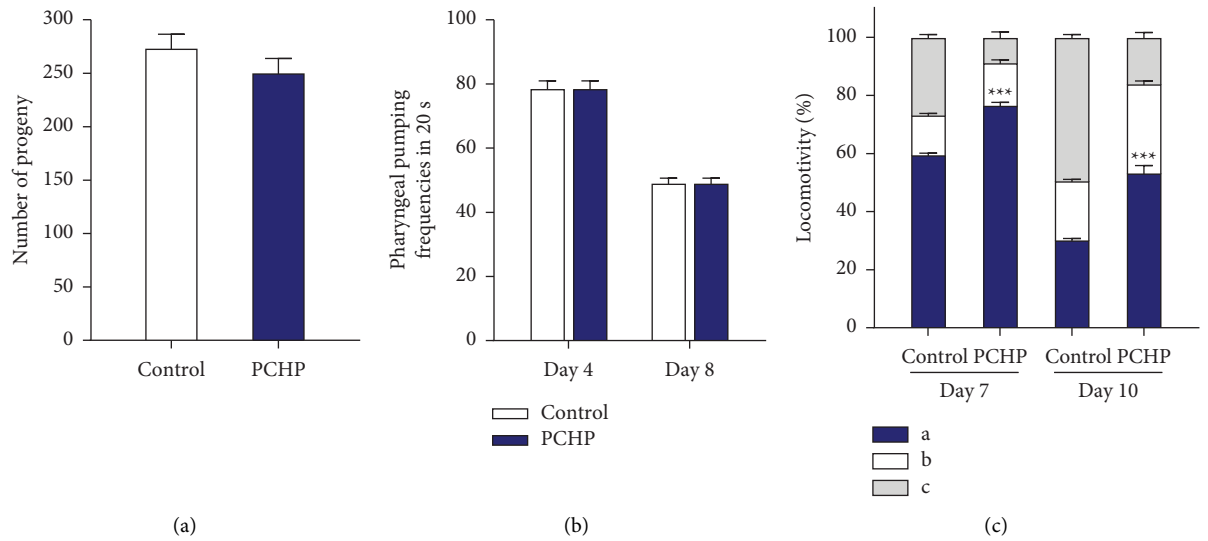

Figure 4: Effects of 5.0 mg/mL PCHP on the number of offspring in *C. elegans* (a), the pharyngeal pumping rate on days 4 and 8 of adulthood (b), and the locomotivity on days 7 and 10 of adulthood (c). *** $p < 0.001$ indicates significant differences when compared to the control group.

Excessive ROS are generated as byproducts of aerobic metabolism and are closely associated with the damage of many macromolecules, including DNA, lipids, and proteins. Previous studies have demonstrated that PRPs have strong free radical scavenging ability and reducing capacity [30]. As one of the main bioactive components in Polygonati rhizoma, polysaccharides showed fantastic characteristic on antioxidant activity [35–37]. Our results demonstrated that PCHP significantly reduced the levels of ROS and lipofuscin in *C. elegans*. This suggests that PCHP can significantly improve the antioxidant properties of *C. elegans* and delay senescence, which might be attributed to its great free radical scavenging ability and reducing capacity [30].

3.5. Effects of PCHP on the Resistance of Wild-TypeC. elegans to Stress. To investigate whether PCHP could improve lifespan extension in *C. elegans* exposed to environmental stress, the survival rates of wild-type N2 worms were examined under heat stress, UV irradiation, and oxidative stress induced by paraquat. PCHP (5.0 mg/mL) enhanced the resistance of worms to heat stress (Figures 6(a) and 6(d)). The mean lifespan increased to 7.33 ± 0.47 h compared with that of the control group (5.00 ± 0.21 h), and PCHP significantly extended the mean lifespan of worms by 46.67%. After exposure to UV irradiation, the mean lifespan of the control group was determined to be 10.40 ± 0.14 days, whereas that of the groups treated with PCHP was dramatically increased

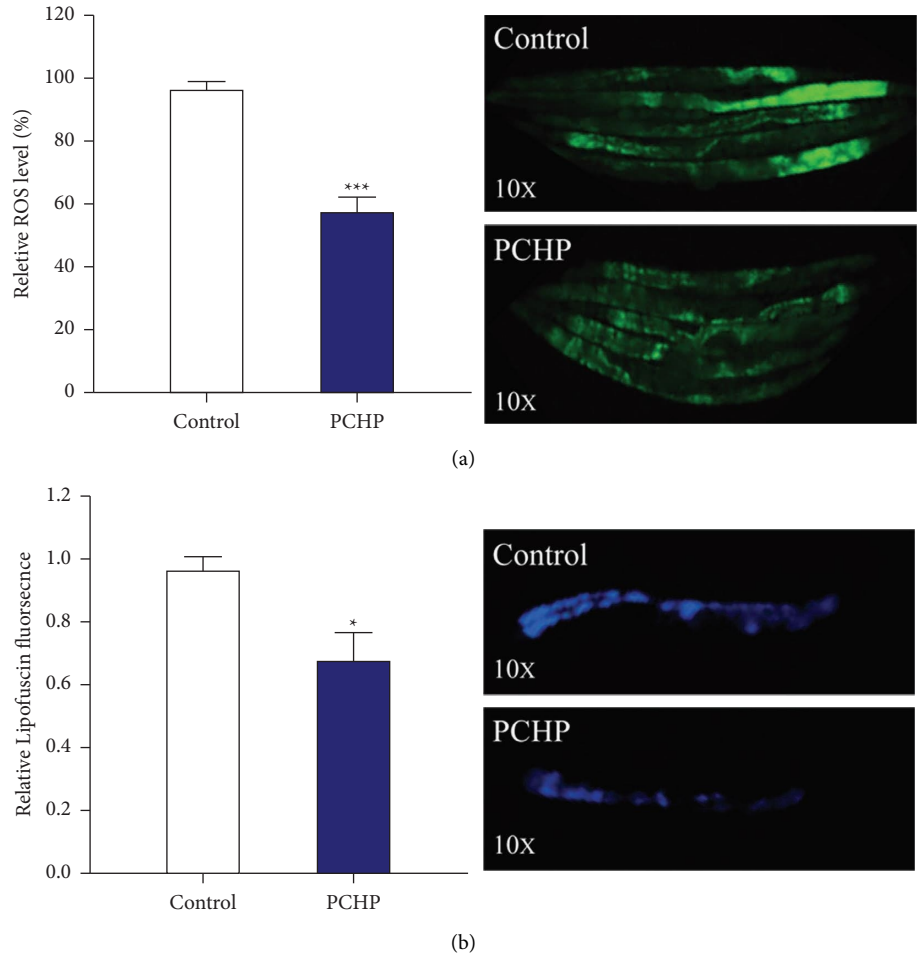

Figure 5: On day 8 of adulthood, the accumulation of ROS (a) and lipofuscin (b) of wild-type N2 C. elegans with or without 5.0 mg/mL PCHP treatment were determined. Data are expressed as the mean ± SD of three independent experiments. $^{*}p < 0.05$, and $^{***}p < 0.001$ indicate significant differences when compared to the control group.

to 13.05 ± 1.03 days with a prolonging percentage of 25.48% (Figures 6(b) and 6(e)). Simultaneously, worms treated with PCHP had a significant mean lifespan extension (PCHP group: 6.92 ± 0.86 days and control group: 3.98 ± 0.14 days) by 73.64% under paraquat-induced oxidative stress (Figures 6(c) and 6(f)). These results indicated that PCHP can improve the resistance of worms against the damage from environmental stress.

Oxidative stress is characterized by an imbalance between intracellular antioxidant defense activities and the disorder of cellular redox balance. Oxidative stress and the "free radical theory" are commonly used to explain aging [33]. Similarly, the prolongation of the lifespan of worms is accompanied with an increase in their resistance to stress. The stress modulation played an important role in lifespan extension [38]. Based on the above analysis, PCHP showed great advantageous for stress resistance under heat stress, UV irradiation, and oxidative stress. Together with the effect of PCHP on ROS, PCHP could effectively scavenge ROS and mediate lifespan extension in *C. elegans*, which might be attributed to its antioxidant properties that lead to improved tolerance to oxidative and thermal stress. These results suggest that PCHP could extend lifespan though enhancing antioxidant defense system in *C. elegans*.

3.6. Effect of PCHP on the DAF-16::GFP Localization of C. elegans. DAF-16 is an important transcription factor that mediates the longevity and stress resistance in *C. elegans*, and the nuclear localization of DAF-16 is indispensable for activating downstream genes [39, 40]. To further study whether the transcription factor DAF-16 plays a role in the PCHP-mediated longevity of worms, the *TJ356* strain containing GFP::DAF-16 was used to uncover the impact of PCHP on DAF-16 nuclear localization. The results revealed that the cytosolic DAF-16::GFP fraction significantly decreased from 67.33% to 22.67%, whereas the nuclear fraction remarkably increased from 11.67% to 55.67% after treatment with 5.0 mg/mL PCHP compared with the control group (Figure 7). The intermediate fraction showed no significant

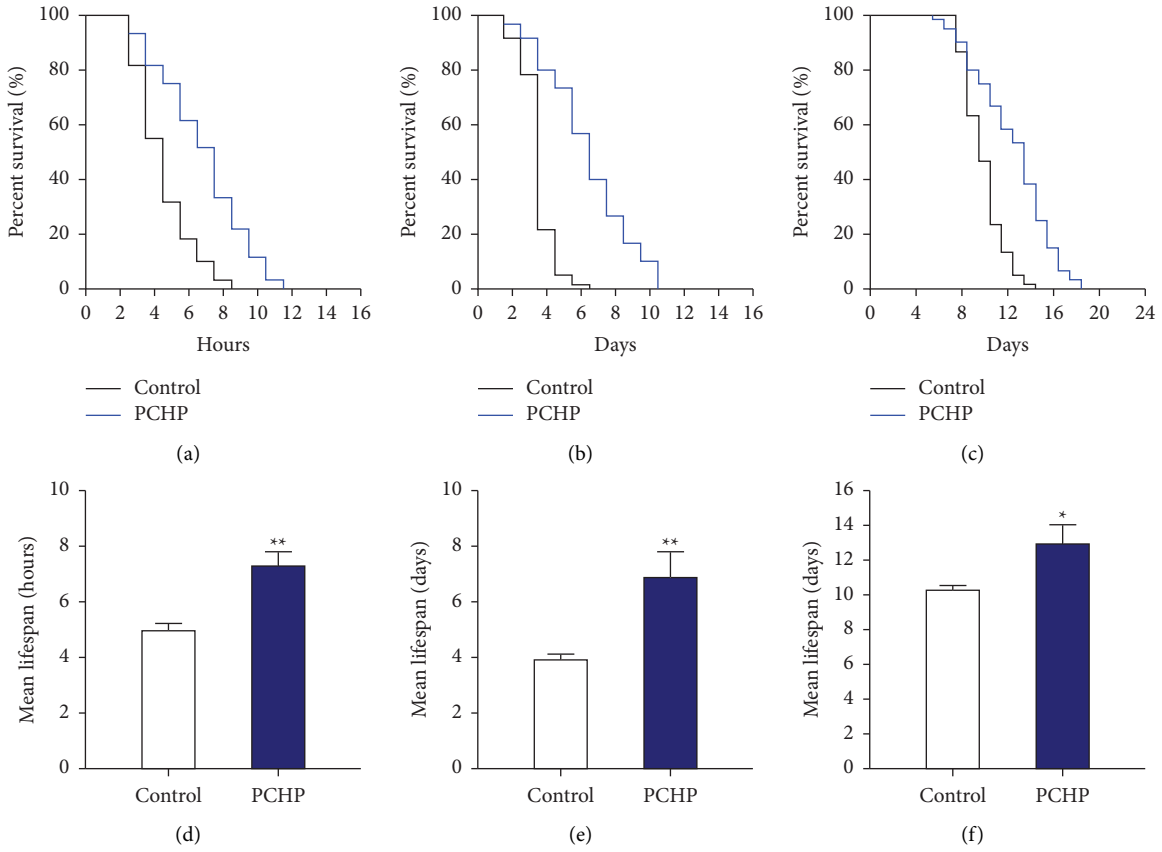

Figure 6: The survival rate curves and the mean lifespan of wild-type N2 *C. elegans* under heat shock (a, d), UV damage (b, e), and paraquat-induced oxidative stress (c, f) after treatment with or without 5.0 mg/mL PCHP. Data are expressed as mean ± SD of three independent experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$ indicate significant differences when compared to the control group.

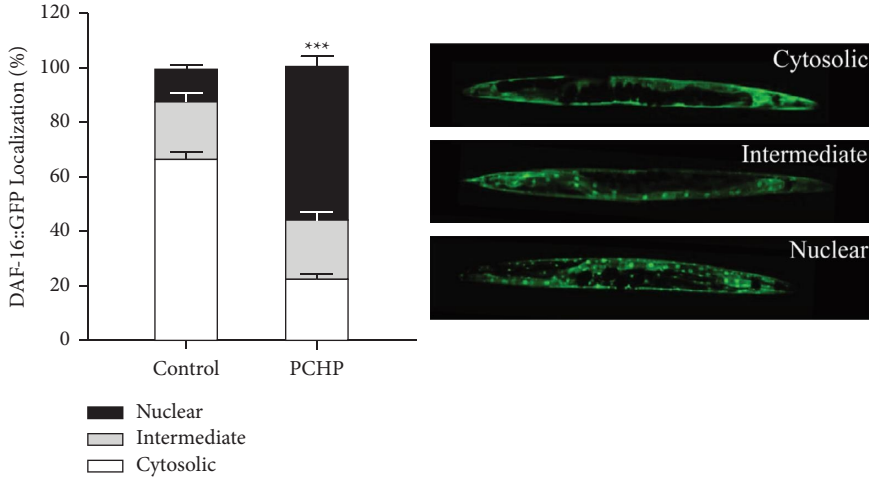

Figure 7: Quantification of DAF-16::GFP accumulation in the nuclei of worms treated with or without 5.0 mg/mL PCHP. Data are expressed as the mean ± SD of three independent experiments. $^{***}p < 0.001$ indicates significant differences when compared to the control group.

difference between the two groups. It suggested that PCHP could alter the distribution of DAF-16, which made the DAF-16 in the cytosol translocate into nuclei. The translocation of DAF-16 can activate downstream genes that mediate stress resistance and aging. Thus, DAF-16 is thought to be involved in the PCHP-mediated longevity of worms.

3.7. Effect of PCHP on the mRNA Expression of Aging-Related and Stress-Related Genes in C. elegans. After confirming the effects of PCHP on the longevity and stress resistance in *C. elegans* in order to further explore the role of insulin signaling in the PCHP-mediated lifespan extension, the expression levels of genes involved in longevity and stress resistance were evaluated in this study. The results demonstrated that 5.0 mg/mL PCHP influenced the expression of *daf-2*, *daf-16*, *sod-3*, *skn-1*, and *sir-2.1*. The expression of *sod-3*, *skn-1*, and *sir-2.1* was significantly upregulated after PCHP treatment, particularly that *sod-3*, by 2.70-, 0.89-, and 0.78-fold, respectively, compared with that of the control group (Figure 8). However, the expression levels of *daf-2* and *daf-16* were upregulated by 0.09- and 0.13-fold, respectively, with no statistically significant difference between the two groups. Overall, PCHP differentially regulated the expression of aging- and stress-related genes.

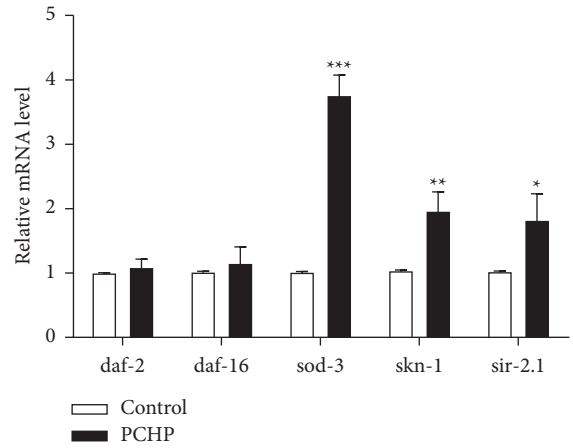

Figure 8: Gene expression of *daf-2*, *daf-16*, *sod-3*, *skn-1*, and *sir-2.1* in wild-type N2 *C. elegans* treated with or without 5.0 mg/mL PCHP. Data are expressed as mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ indicate significant differences when compared to the control group.

3.8. PCHP Extends the Lifespan of C. elegans via DAF-16/SIR-2.1/SKN-1. The IIS pathway, implicated in processes such as metabolism, growth, longevity, and oxidative responses, is able to modulate the lifespan in *C. elegans*. It is regulated by the insulin/insulin-like growth factor receptor DAF-2 via a conserved AGE-1/phosphoinositide 3-kinase (PI3K)/Akt kinase-1/2 cascade, which terminates the regulation of the transcription factor DAF-16 [41, 42]. DAF-16 is a major target activated by decreased insulin-like signaling, which is involved in lifespan and stress resistance [43].

To determine whether the PCHP mediated the longevity of worms via insulin signaling pathways, the lifespan of *daf-2 (e1370)*, *age-1 (hx546)*, and *daf-16 (mu86)* mutants treated with PCHP was explored (Figure 9). The lifespan of the *daf-2* mutant, a long-lived insulin-like signaling receptor mutant, was not extended by PCHP (5.0 mg/mL). The lifespan of the *daf-2* mutant treated with PCHP was significantly decreased by 30.06% compared to that of the control group (Figure 9(a) and Table 3). This might be related to the inactivation of some genes induced by PCHP, which causes the greatest acceleration in *daf-2* aging [44]. Furthermore, the lifespan extension effects of PCHP were absent in the loss-of-function*age-1 (hx546)* and *daf-16 (mu86)* mutants (Figures 9(b) and 9(c) and Table 3). This indicates that PCHP-mediated longevity requires an IIS pathway. Although the expression of daf-*16* exhibited no significant increase in mRNA levels, the effect of PCHP on the translocation of DAF-16 from the cytosol to the nucleus was notable, indicating that changes at the protein level might occur, which requires further investigation. The *sod-3*, a downstream target gene of *daf-16* involved in the antioxidant stress response, was remarkably upregulated after treatment. The effects of PCHP on the translocation of DAF-16 and upregulated expression of *sod-3* confirmed that *DAF*-16 plays an important role in the PCHP-regulated stress response. Taken together, the PCHP-mediated longevity and stress resistance of *C. elegans* are attributed to the IIS pathway.

The transcription factor SKN-1, a well-known antiaging and longevity factor, plays a critical role in the antiaging and stress resistance of *C. elegans* [45, 46]. It is activated to regulate the expression of various genes encoding antioxidant response proteins. To investigate whether PCHP-mediated antiaging requires SKN-1, the lifespan of *skn-1 (zu135)* mutants was measured. The results showed no notable differences after PCHP treatment (Figure 9(d) and Table 3), indicating that PCHP-mediated antiaging is dependent on SKN-1. In response to the increase in intracellular ROS, as a part of the cellular defense system against oxidative stress, the oxidative stress-response factor SKN-1 protects the worms from oxidative damage [47, 48]. The qPCR expression of the *skn-1* gene, which is responsible for antioxidant activity, was analyzed in wild-type N2 *C. elegans* treated with PCHP to further validate this effect. Compared to the control group, the expression of *skn-1* was significantly increased in *C. elegans* treated with PCHP. Therefore, SKN-1 activates the oxidative stress defense mechanism involved in PCHP-mediated antiaging effects.

In addition to DAF-16 and SKN-1, other factors are involved in the regulation of the lifespan in *C. elegans*. SIR-2.1, a stress response regulatory transcription factor, encodes an NAD-dependent histone deacetylase. This factor may affect the lifespan of *C. elegans* by regulating the transcription of stress-related genes [18, 46]. It has been suggested that SIR-2.1 is likely to activate DAF-16 in a 14-3-3-dependent manner [49]. To examine whether SIR-2.1 is involved in PCHP-mediated longevity, a lifespan assay in the *sir-2.1 (ok434)* mutant was performed. The results showed that the effect of 5.0 mg/mL PCHP on lifespan extension was entirely disappeared in the *sir-2.1 (ok434)* mutant (Figure 9(e) and Table 3), indicating that PCHP extended the lifespan of *C. elegans* in a *sir-2.1*-dependent manner. SIR-2.1

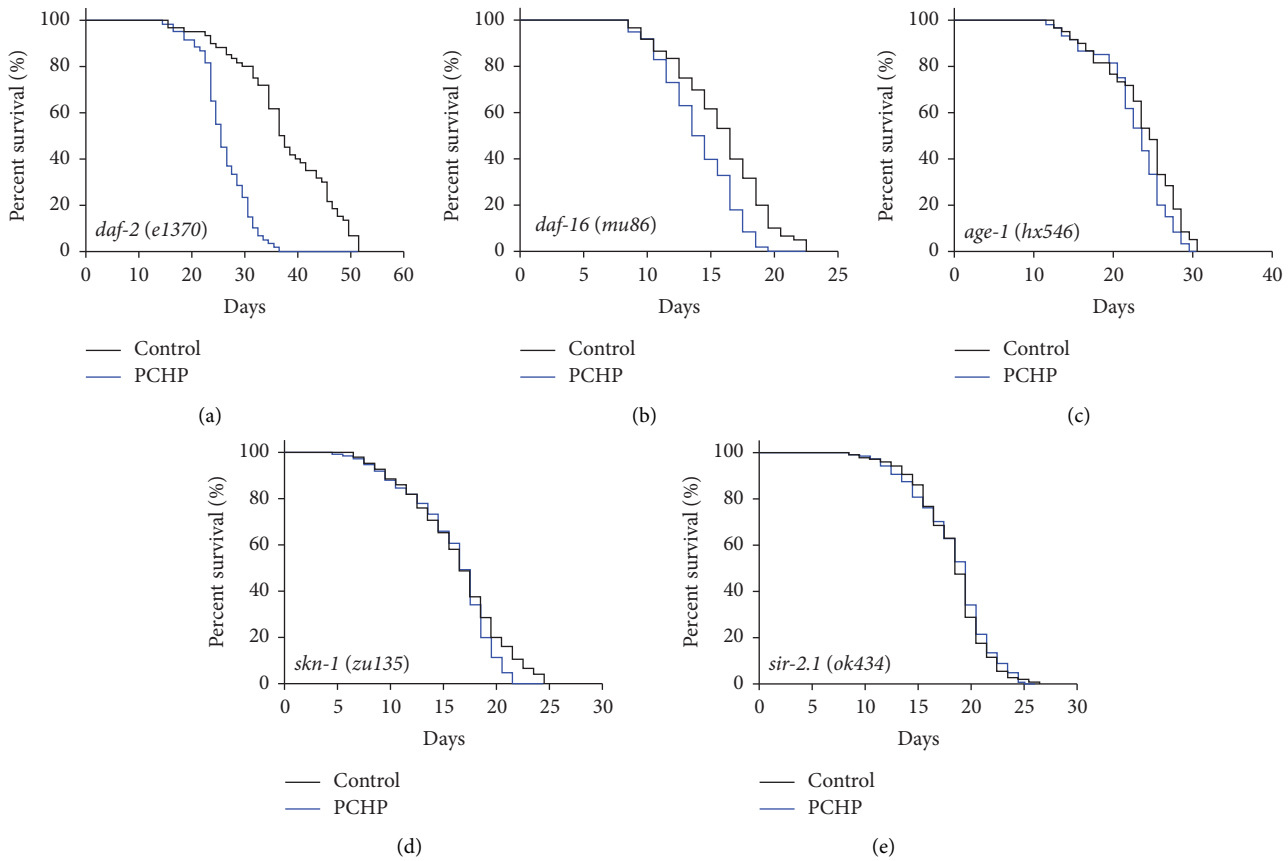

Figure 9: The survival rate curves of mutants *daf-2* (a), *daf-16* (b), *age-1* (c), *skn-1* (d), and *sir-2.1* (e), treated with or without 5.0 mg/mL PCHP at 20°C.

could improve resistance to oxidative stress, and its influence on lifespan extension is needed for the functional presence of SIR-2.1 [50]. It was conjectured that the expression of defense genes could be upregulated after treatment; therefore, the influence of PCHP treatment on the expression of the *sir-2.1* gene was assessed in this study. Compared to that in the control group, the *sir-2.1* gene was significantly upregulated in wild-type N2 *C. elegans* after treatment with PCHP, indicating that SIR-2.1 also plays an important role in the lifespan extension of *C. elegans* by PCHP treatment.

These findings suggest that the lifespan extension and stress resistance mediated by PCHP are associated with *DAF*-16, SIR2.1, and SKN-1.

PRPs have strong antioxidant activity and can be used to treat some diseases caused by an imbalance in the oxidation–reduction processes in organisms [9, 30]. Although studies have demonstrated that PRPs have antiaging effects, the underlying mechanisms have rarely been investigated. Furthermore, some studies have investigated the antiaging effects of PRPs after wine stewing [30]; however, the antiaging effects and mechanism of action of PCHP processed by steam baking remain unclear. Our results demonstrate that the antiaging effects of PCHP after steam baking processing might be associated with its antioxidant activity. It exhibited better antiaging activity than the polysaccharides from raw or wine-stewed Polygonati rhizoma. Studies showed that PCHP could reduce the levels of ROS induced by oxidative stress via regulating NRF2/HO-1 pathway [37, 38], which can explain that the PCHP mediate antiaging. *P. sibiricum* polysaccharides were found to have great effects on improving the immune function and can be distributed in most tissues of rats after administration [51, 52]. Furthermore, it could regulate the gut microbiota in rats. These effects of polysaccharide might be related to PCHP-mediated lifespan extension in *C. elegans*. However, processing can change polysaccharide characteristics, such as the type, quantity, and sequence of monosaccharides. Therefore, processing cycles that are appropriate for antiaging need to be investigated further. Reportedly, this is the first systematic study of antiaging and stress resistance on PCHP in *C. elegans*, which is helpful in promoting functional food research on Polygonati rhizoma, especially in decelerating aging.

4. Conclusions

Overall, this study demonstrated that PCHP could decelerate aging in *C. elegans*, enhance stress resistance, and benefit exercise behavior by decreasing the levels of ROS and

lipofuscin and increasing the nuclear translocation of DAF-16. PCHP significantly upregulated the mRNA expression of *sod-3*, *skn-1*, and *sir-2.1* in wild-type*C. elegans* treated with 5.0 mg/mL PCHP. This suggests that antiaging and antioxidative stress effects of PCHP may be dependent on DAF-16/SIR2.1/SKN-1. Our study provides an indispensable basis for understanding the potential antiaging mechanism of polysaccharides from *P. cyrtonema*. Furthermore, these findings demonstrate the beneficial effects of Polygonati rhizoma on facing aging and provide a new perspective on investigation of polysaccharides from nine cycles of steam baking processing for Polygonati rhizoma on antiaging.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Wei Wang and Ying Yang contributed equally to this work.

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Supplementary Materials

Figure S1: the standard curves for detection of monosaccharide-derivatives. (*Supplementary Materials*)

References

[1] W. Wujisguleng, Y. Liu, and C. Long, "Ethnobotanical review of food uses of Polygonatum (Convallariaceae) in China," *Acta Societatis Botanicorum Poloniae*, vol. 81, no. 4, pp. 239–244, 2012.

[2] H. Chen, S. S. Feng, Y. J. Sun, Z. Y. Hao, W. S. Feng, and X. K. Zheng, "Advances in studies on chemical constituents of three medicinal plants from Polygonatum mill and their pharmacological activities," *Chinese Traditional and Herbal Drugs*, vol. 46, no. 15, pp. 2329–2338, 2015.

[3] W. Zhao, Y. Zhao, and Y. Tseng, "Research progress on pharmacological effects of Polygonati Rhizoma," *Chinese Traditional and Herbal Drugs*, vol. 49, no. 18, pp. 4439–4445, 2018.

[4] W. X. Wang, X. Zhang, X. L. T. Dabu et al., "Analysis of chemical constituents from Polygonatum cyrtonema after "Nine-Steam-Nine-Bask" processing," *Phytochemistry Letters*, vol. 29, pp. 35–40, 2019.

[5] K. Ma, X. F. Huang, and L. Y. Kong, "Steroidal saponins from Polygonatum cyrtonema," *Chemistry of Natural Compounds*, vol. 49, no. 5, pp. 888–891, 2013.

[6] L. R. Sun, X. Li, and S. X. Wang, "Two new alkaloids from the rhizome of Polygonatum sibiricum," *Journal of Asian Natural Products Research*, vol. 7, no. 2, pp. 127–130, 2005.

[7] L. Li, B. Y. Liao, K. Thakur, J. G. Zhang, and Z. J. Wei, "The rheological behavior of polysaccharides sequential extracted from Polygonatum cyrtonema Hua," *International Journal of Biological Macromolecules*, vol. 109, pp. 761–771, 2018.

[8] P. Zhao, C. Zhao, X. Li et al., "The genus Polygonatum: a review of ethnopharmacology, phytochemistry and pharmacology," *Journal of Ethnopharmacology*, vol. 214, pp. 274–291, 2018.

[9] L. Li, K. Thakur, B. Y. Liao, J. G. Zhang, and Z. J. Wei, "Antioxidant and antimicrobial potential of polysaccharides sequentially extracted from Polygonatum cyrtonema Hua," *International Journal of Biological Macromolecules*, vol. 114, pp. 317–323, 2018.

[10] B. Fan, G. Wei, X. Gan et al., "Study on the varied content of Polygonatum cyrtonema polysaccharides in the processing of steaming and shining for nine times based on HPLC-MS/MS and chemometrics," *Microchemical Journal*, vol. 159, Article ID 105352, 2020.

[11] Y. Luan, Y. Jiang, R. Huang et al., "Polygonati rhizoma polysaccharide prolongs lifespan and healthspan in *Caenorhabditis elegans*," *Molecules*, vol. 28, no. 5, p. 2235, 2023.

[12] Y. He, Z. Chen, X. Nie et al., "Recent advances in polysaccharides from edible and medicinal Polygonati rhizoma: from bench to market," *International Journal of Biological Macromolecules*, vol. 195, pp. 102–116, 2022.

[13] P. Zhao, H. Zhou, C. Zhao et al., "Purification, characterization and immunomodulatory activity of fructans from Polygonatum odoratum and P. cyrtonema," *Carbohydrate Polymers*, vol. 214, pp. 44–52, 2019.

[14] W. Wu, N. Huang, J. Huang et al., "Effects of the steaming process on the structural properties and immunological activities of polysaccharides from Polygonatum cyrtonema," *Journal of Functional Foods*, vol. 88, Article ID 104866, 2022.

[15] Z. Chen, B. Zhu, Z. Chen et al., "Effects of steam on polysaccharides from Polygonatum cyrtonema based on saccharide mapping analysis and pharmacological activity assays," *Chinese Medicine*, vol. 17, no. 1, p. 97, 2022.

[16] S. Zhu, P. Liu, W. Wu et al., "Multi-constituents variation in medicinal crops processing: investigation of nine cycles of steam-sun drying as the processing method for the rhizome of Polygonatum cyrtonema," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 209, Article ID 114497, 2022.

[17] P. Shen, Y. Yue, J. Zheng, and Y. Park, "*Caenorhabditis elegans*: a convenient in vivo model for assessing the impact of food bioactive compounds on obesity, aging, and Alzheimer's disease," *Annual Review of Food Science and Technology*, vol. 9, pp. 1–22, 2018.

[18] M. L. Sun, X. Y. Chen, J. J. Cao, X. H. Cui, and H. B. Wang, "Polygonum multiflorum Thunb extract extended the lifespan and healthspan of *Caenorhabditis elegans* via DAF-16/SIR-2.1/SKN-1," *Food & Function*, vol. 12, no. 18, pp. 8774–8786, 2021.

[19] Y. Ge, H. Chen, J. Wang et al., "Naringenin prolongs lifespan and delays aging mediated by IIS and MAPK in *Caenorhabditis elegans*," *Food & Function*, vol. 12, no. 23, pp. 12127–12141, 2021.

[20] Y. Yuan, N. Kang, Q. Li, Y. Zhang, Y. Liu, and P. Tan, "Study of the effect of neutral polysaccharides from rehmannia

glutinosa on lifespan of *Caenorhabditis elegans*," *Molecules*, vol. 24, no. 24, p. 4592, 2019.

[21] X. Guo, J. Luo, J. Qi et al., "The role and mechanism of polysaccharides in anti-aging," *Nutrients*, vol. 14, no. 24, p. 5330, 2022.

[22] Q. Wang, J. Wu, J. Huang et al., "Cremastra appendiculata polysaccharides improve stress resistance and prolong the lifespan of *Caenorhabditis elegans* via daf-16 in the insulin signaling pathway," *International Journal of Biological Macromolecules*, vol. 229, pp. 496–506, 2023.

[23] A. M. Staub, "Removeal of protein-Sevag method," *Methods in Carbohydrate Chemistry*, vol. 5, pp. 5-6, 1965.

[24] C. Y. Liu, D. J. Hu, H. Zhu et al., "Preparation, characterization and immunoregulatory activity of derivatives of polysaccharide from Atractylodes lancea (Thunb.) DC," *International Journal of Biological Macromolecules*, vol. 216, pp. 225–234, 2022.

[25] B. Liu, Z. Z. Shang, Q. M. Li et al., "Structural features and anti-gastric cancer activity of polysaccharides from stem, root, leaf and flower of cultivated Dendrobium huoshanense," *International Journal of Biological Macromolecules*, vol. 143, pp. 651–664, 2020.

[26] L. A. Herndon, P. J. Schmeissner, J. M. Dudaronek et al., "Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*," *Nature*, vol. 419, no. 6909, pp. 808–814, 2002.

[27] J. P. Ke, J. Y. Yu, B. Gao et al., "Two new catechins from Zijuan green tea enhance the fitness and lifespan of *Caenorhabditis elegans* via insulin-like signaling pathways," *Food & Function*, vol. 13, no. 18, pp. 9299–9310, 2022.

[28] J. Jin, J. Lao, R. Zhou et al., "Simultaneous identification and dynamic analysis of saccharides during steam processing of Rhizomes of Polygonatum cyrtonema by HPLC-QTOF-MS/MS," *Molecules*, vol. 23, no. 11, p. 2855, 2018.

[29] Z. Wang, R. Xie, and B. Wang, "Comprehensive evaluation and anti-inflammatory activity of "Zhi" polygonatum cyrtonema produced by the classical steaming approach," *Pharmacological Research-Modern Chinese Medicine*, vol. 6, Article ID 100229, 2023.

[30] S. Wang, F. He, H. Wu et al., "Health-promoting activities and associated mechanisms of Polygonati rhizoma polysaccharides," *Molecules*, vol. 28, no. 3, p. 1350, 2023.

[31] C. Huang, C. Xiong, and K. Kornfeld, "Measurements of age-related changes of physiological processes that predict lifespan of *Caenorhabditis elegans*," *Proceedings of the National Academy of Sciences*, vol. 101, no. 21, pp. 8084–8089, 2004.

[32] H. Hui, A. Xin, H. Cui et al., "Anti-aging effects on *Caenorhabditis elegans* of a polysaccharide, O-acetyl glucomannan, from roots of Lilium davidii var. unicolor Cotton," *International Journal of Biological Macromolecules*, vol. 155, pp. 846–852, 2020.

[33] D. Harman, "Aging: a theory based on free radical and radiation chemistry," *Journal of Gerontology*, vol. 11, no. 3, pp. 298–300, 1956.

[34] Y. Hu, Y. Tang, Z. Zhang et al., "Recent advances in polysaccharides from the genus Polygonatum: isolation, structures, bioactivities, and application," *Food Hydrocolloids*, vol. 140, Article ID 108634, 2023.

[35] S. Wang, G. Li, X. Zhang et al., "Structural characterization and antioxidant activity of Polygonatum sibiricum polysaccharides," *Carbohydrate Polymers*, vol. 291, Article ID 119524, 2022.

[36] J. Li, X. Wang, R. Zhou et al., "Polygonatum cyrtonema Hua polysaccharides protect BV2 microglia relief oxidative stress and ferroptosis by regulating NRF2/HO-1 pathway," *Molecules*, vol. 27, no. 20, p. 7088, 2022.

[37] H. Teng, Y. Zhang, C. Jin et al., "Polysaccharides from steam-processed Polygonatum cyrtonema Hua protect against d-galactose-induced oxidative damage in mice by activation of Nrf2/HO-1 signaling," *Journal of the Science of Food and Agriculture*, vol. 103, no. 2, pp. 779–791, 2023.

[38] C. Wu, J. Liu, J. Ma, Q. Yan, and Z. Jiang, "Neoagarotetraose extends the lifespan of *Caenorhabditis elegans* through AMPK mediated signaling pathways and activation of autophagy," *Journal of Functional Foods*, vol. 77, Article ID 104341, 2021.

[39] K. Lin, H. Hsin, N. Libina, and C. Kenyon, "Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling," *Nature Genetics*, vol. 28, no. 2, pp. 139–145, 2001.

[40] S. S. Lee, S. Kennedy, A. C. Tolonen, and G. Ruvkun, "DAF-16 target genes that control *C. elegans*life-span and metabolism," *Science*, vol. 300, no. 5619, pp. 644–647, 2003.

[41] A. Zečić and B. P. Braeckman, "DAF-16/FoxO in *Caenorhabditis elegans* and its role in metabolic remodeling," *Cells*, vol. 9, no. 1, p. 109, 2020.

[42] T. Xu, M. Tao, R. Li, X. Xu, S. Pan, and T. Wu, "Longevity-promoting properties of ginger extract in *Caenorhabditis elegans* via the insulin/IGF-1 signaling pathway," *Food & Function*, vol. 13, no. 19, pp. 9893–9903, 2022.

[43] M. Uno, Y. Tani, M. Nono et al., "Neuronal DAF-16-to-intestinal DAF-16 communication underlies organismal lifespan extension in *C. elegans*," *iScience*, vol. 24, no. 7, Article ID 102706, 2021.

[44] A. V. Samuelson, C. E. Carr, and G. Ruvkun, "Gene activities that mediate increased life span of *C. elegans*insulin-like signaling mutants," *Genes & Development*, vol. 21, no. 22, pp. 2976–2994, 2007.

[45] J. M. Tullet, M. Hertweck, J. H. An et al., "Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*," *Cell*, vol. 132, no. 6, pp. 1025–1038, 2008.

[46] J. H. An and T. K. Blackwell, "SKN-1 links *C. elegans* mesendodermal specification to a conserved oxidative stress response," *Genes & Development*, vol. 17, no. 15, pp. 1882–1893, 2003.

[47] H. Liu, Y. Wang, W. Zhang et al., "Lentinan extends lifespan and increases oxidative stress resistance through DAF-16 and SKN-1 pathways in *Caenorhabditis elegans*," *International Journal of Biological Macromolecules*, vol. 202, pp. 286–295, 2022.

[48] S. Dayalan Naidu, R. V. Kostov, and A. T. Dinkova-Kostova, "Transcription factors Hsf1 and Nrf2 engage in crosstalk for cytoprotection," *Trends in Pharmacological Sciences*, vol. 36, no. 1, pp. 6–14, 2015.

[49] A. Berdichevsky, M. Viswanathan, H. R. Horvitz, and L. Guarente, "*C. elegans* SIR-2.1 interacts with 14-3-3 proteins to activate DAF-16 and extend life span," *Cell*, vol. 125, no. 6, pp. 1165–1177, 2006.

[50] Y. Zhang, Q. Shi, W. Jiang et al., "Comparison of the chemical composition and antioxidant stress ability of polysaccharides

from Auricularia auricula under different drying methods," *Food & Function*, vol. 13, no. 5, pp. 2938–2951, 2022.

[51] J. Bi, C. Zhao, W. Jin, Q. Chen, B. Fan, and C. Qian, "Study on pharmacokinetics and tissue distribution of Polygonatum sibiricum polysaccharide in rats by fluorescence labeling," *International Journal of Biological Macromolecules*, vol. 215, pp. 541–549, 2022.

[52] T. Lu, Z. Guo, X. Li et al., "Effects of wine processed Polygonatum polysaccharides on immunomodulatory effects and intestinal microecology in mice," *Quality Assurance and Safety of Crops & Foods*, vol. 15, no. 2, pp. 161–174, 2023.